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Original Article

Developmental regulation of p53-dependent radiation-induced thymocyte apoptosis in mice

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Summary

The production of T cell receptor αβ⁺ (TCRαβ⁺) T lymphocytes in the thymus is a tightly regulated process that can be monitored by the regulated expression of several surface molecules, including CD4, CD8, cKit, CD25 and the TCR itself, after TCR genes have been assembled from discrete V, D (for TCR-β) and J gene segments by a site-directed genetic recombination. Thymocyte differentiation is the result of a delicate balance between cell death and survival: developing thymocytes die unless they receive a positive signal to proceed to the next stage. This equilibrium is altered in response to various physiological or physical stresses such as ionizing radiation, which induces a massive p53-dependent apoptosis of CD4⁺CD8⁺ double-positive (DP) thymocytes. Interestingly, these cells are actively rearranging their TCR-α chain genes. To unravel an eventual link between V(D)J recombination activity and thymocyte radio-sensitivity, we analysed the dynamics of thymocyte apoptosis and regeneration following exposure of wild-type and p53-deficient mice to different doses of γ-radiation. p53-dependent radiosensitivity was already found to be high in immature CD4⁻CD8⁻ (double-negative, DN) cKit⁺CD25⁺ thymocytes, where TCR-β gene rearrangement is initiated. However, TCR-αβ⁻CD8⁺ immature single-positive thymocytes, an actively cycling intermediate population between the DN and DP stages, are the most radio-sensitive cells in the thymus, even though their apoptosis is only partially p53-dependent. Within the DP population, TCR-αβ⁺ thymocytes that completed TCR-α gene recombination are more radio-resistant than their TCR-αβ⁻ progenitors. Finally, we found no correlation between p53 activation and thymocyte sensitivity to radiation-induced apoptosis.

Keywords: apoptosis, p53, radiation, thymocyte differentiation

Introduction

T cell receptor-$\alpha\beta^+$ (TCR-$\alpha\beta^+$) T lymphocyte development is the result of a complex programme initiated in the thymus by blood-borne haematopoietic stem cell-derived progenitors. The regulated expression of several surface molecules, including the TCR chains, marks the progression of developing thymocytes through the different maturation stages. The most immature expresses neither CD4 nor CD8 ($CD4^-CD8^-$, DN). Within this population, thymocytes progress from the $CD44^+cKit^+CD25^-$ (DN1), to the $CD44^+cKit^+CD25^+$ (DN2), $CD44^-cKit^-CD25^+$ (DN3) and $cD44^-cKit^-CD25^-$ (DN4) subpopulations as they mature [1,2]. TCR-β expression in a pre-TCR complex on DN3 thymocytes is required for differentiation to the DN4 stage [3], and then through the rapidly cycling $CD8^+$TCR-$\alpha\beta^-$ immature single-positive (ISP) stage [4,5], to the $CD4^+CD8^+$ double-positive (DP) population. DP thymocytes first express only low levels of TCR-$\alpha\beta$, until their antigenic specificity is tested through positive and negative selection [6–8]. At that stage, they up-regulate TCR-$\alpha\beta$ expression and transiently express CD69 [9,10] before being sorted into mature TCR-$\alpha\beta^+CD4^+$ and TCR-$\alpha\beta^+CD8^+$ single-positive (SP) thymocytes and exported to the periphery. At each step of differentiation, thymocytes must receive a positive cue, first from cell–cell interactions and cytokine signalling and then from pre-TCR- and TCR-derived signals, to progress to the next stage, otherwise they die [11–14]. Thymocytes are extremely sensitive to various kinds of physiological and physical stresses that induce a massive thymic involution [15].

Before being expressed, the TCR chain genes must be rearranged from discrete V, D (for TCR-β) and J gene segments by a site-directed genetic recombination mechanism. During V(D)J recombination, the lymphoid-specific recombination-activating gene (RAG1)/RAG2 introduces DNA double-stranded breaks (DSBs) at the border of each gene [16–18], which are then repaired by non-homologous end joining [19]. The creation of these DSBs, even in this physiological context, activates some of the actors of the checkpoints elicited in response to genotoxic stress [20–22]. This 'pre-activation' state could explain the well-documented high p53-dependent radio-sensitivity of DP thymocytes [23–25]. To unravel an eventual link between V(D)J recombination and p53-dependent thymocyte radio-sensitivity, we analysed the dynamics of thymocyte apoptosis following exposure of wild-type (WT) and p53-deficient mice to different doses of γ-radiation. We found that before reaching the DP compartment, DN2, in which TCR-β gene rearrangement is initiated [26–28], and ISP thymocytes are already extremely radio-sensitive. ISP apoptosis can be observed as early as 3 h post-exposure and is only partially p53-dependent. In the DP population, TCR selection marks the end of the high sensitivity to IR exposure. Unexpectedly, the highest susceptibility to p53-dependent radiation-induced apoptosis does not depend upon the higher level of p53.

Materials and methods

Mice

C57BL/6 WT and p53-deficient [23–25] mice bred under specific pathogen-free conditions in the animal facility of CEA-Grenoble were exposed to a ^{60}Co source with a dose rate of 2 Gy/min in the Anemome irradiator of the ARC-Nucleart Facility (Grenoble, France). Mice were aged between 7 and 13 weeks at the time of irradiation. These experiments were performed in accordance with French regulations.

Fluorescence activated cell sorter (FACS) analysis

Single-cell thymocyte suspensions were prepared by dissociation of thymi for 30 min at 37°C in collagenase B (2 mg/ml) and DNase I (0·4 mg/ml) in phosphate-buffered saline (PBS)–10% fetal calf serum (FCS) [29]. Labelling was performed as described [30]. Data were acquired on a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and analysed with CellQuest software. Antibodies used in this study were anti-CD4 (GK1·5 or H129·19), anti-CD8 (53–6·7), anti-TCR-β (H57-597), anti-CD69 (H1·2F3), anti-CD3ε (145-2C.11), anti-cKit (2B8), anti-CD44 (IM7) and anti-CD25 (7D4) conjugated to fluorescein isothiocyanate (FITC), Cy-chrome, phycoerythin (PE), allophycocyanin (APC) or biotin. Biotinylated antibodies were revealed with APC-coupled streptavidin. All reagents were from BD Pharmingen (San Diego, CA, USA).

Immunofluorescence staining and confocal microscopy

Thymocytes were labelled with APC-conjugated anti-CD4 and biotinylated anti-CD8 as above and biotin was detected with Alexa 546-coupled streptavidin (Molecular Probes, Eugene, OR, USA). Thymocytes were then attached to poly-L-lysine-coated slides, fixed and permeabilized as described [31]. p53 was detected with a rabbit polyclonal anti-p53 antibody (CM5; Novocastra, Newcastle upon Tyne, UK) revealed by an Alexa 488-conjugated anti-rabbit polyclonal antibody (Molecular Probes). Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) just before mounting the slides in Vectashield (Vector Laboratories, Burlingame, CA, USA). Images were collected under the ×100 objective (numerical aperture = 1·4) of a Leica TSC-P2 confocal microscope in sequential mode for four-colour acquisitions (laser excitation at 488, 543 and 633 nm and a UV source for DAPI). All conditions were compared under identical settings. Images were imported in Adobe Photoshop for figure preparation.

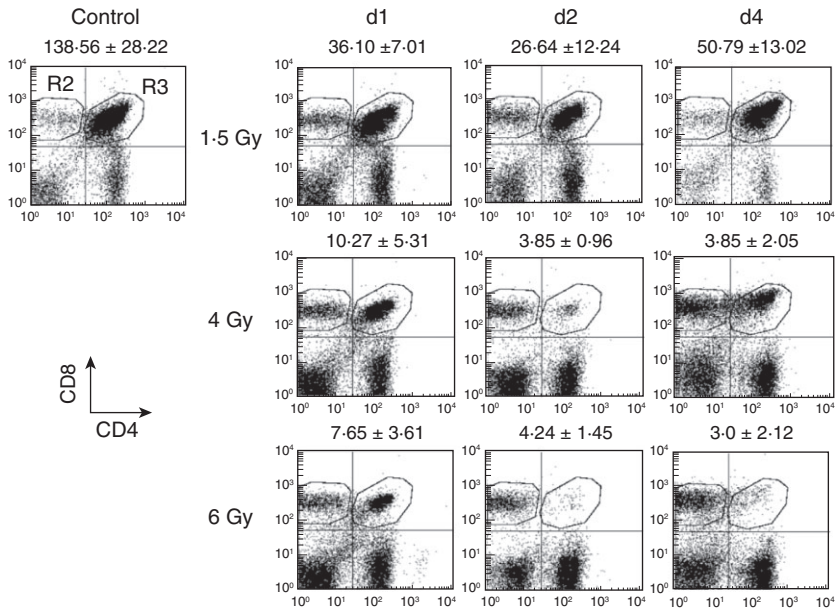

Fig. 1. Thymus involution following radiation exposure. Fluorescence activated cell sorter (FACS) analysis of CD4 and CD8 expression on thymocytes from C57BL/6 mice exposed to 1·5 Gy, 4 Gy or 6 Gy and killed at the indicated times. Live thymocytes were gated on the basis of forward- and side-scatter. The number above each plot is the average (± standard error) number of cells per thymus. The number of mice analysed in each group is as follows: $n = 7$ for control; $n = 3$ for day 1, $n = 4$ for day 2 and $n = 2$ for day 4.

Western blotting

Total cell extracts were prepared from thymocytes by direct lysis of the cells in Laemmli's sample buffer and processed for Western blotting, as described previously [31]. Primary antibodies directed against p53 (CM5; Novocastra), p53 phosphorylated on Ser15 (Cell Signaling, Beverly, MA, USA), γH2AX (Upstate, Billerica, MA, USA) and β tubulin (sc-5274; Santa Cruz Biotechnology, Santa Cruz, CA, USA) were detected with the appropriate horseradish peroxidase-conjugated secondary polyclonal anti-rabbit or anti-mouse, respectively. Membranes were then revealed by chemoluminescence with the enhanced chemiluminescence (ECL) reagent (GE Healthcare, Little Chalfont, UK).

Results

Dynamics of radiation-induced unselected and selected DP thymocyte apoptosis

To analyse the dynamics of the DP thymocyte population following radiation exposure, groups of mice received 1·5, 4 or 6 Gy of γ-radiation (total body irradiation) and were sacrificed 1, 2 and 4 days later to analyse thymic involution. The dose-dependence of the DP thymocyte apoptotic response was evident from the number and phenotype of the surviving thymocytes (Fig. 1). Exposure to 1·5 Gy induces a 75% reduction of thymocyte numbers at day 1 post-exposure. This decrease is slightly accentuated at day 2, but by day 4 the recovery is already apparent. The frequency of DP thymocytes in the thymus of irradiated animals follows this kinetics: the decrease observed at day 1 post-exposure is accentuated from days 1 to 2, before the proportion of DP thymocytes returns to normal at day 4, even if the cellularity is only one-third that of control animals (Fig. 1 and Table 1). Exposure to 4 and 6 Gy produces more severe effects: they induce a decrease of more than 90% of the thymocyte numbers at day 1, which reaches 97% by day 2, with no apparent recovery at day 4. FACS analysis shows a more contrasted situation, and reveals different kinetics for the DP thymocytes. The proportion of DP thymocytes, strongly reduced at day 1, decreases further from days 1 to 2 post-4 and -6 Gy, when it represents fewer than 5% of the living cells, but by day 4 a reprise of DP thymocyte differentiation is visible in irradiated animals, whereas the cell number does not increase. The restoration of the DP pool is very partial (approximately 30 and 7% of thymocytes at 4 and 6 Gy, respectively) when compared to mice exposed to 1·5 Gy (80%) (Table 1).

Table 1. Frequency (± standard error) of the double-negative (DN), double-positive (DP) and single-positive (SP) populations in the thymus of control and irradiated mice shown in Fig. 1.

		1·5 Gy			4 Gy			6 Gy		
	0 Gy	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4
DN	3·38 ± 0·52	5·76 ± 0·50	10·02 ± 1·86	4·71 ± 0·35	12·7 ± 1·22	9·35 ± 1·09	16·65 ± 2·64	15·33 ± 2·08	7·67 ± 0·90	24·32 ± 1·23
DP	83·91 ± 1·10	73·43 ± 1·04	49·11 ± 1·69	80·24 ± 2·76	44·82 ± 4·22	4·68 ± 0·90	31·17 ± 12·40	23·62 ± 1·41	1·56 ± 0·34	7·06 ± 3·55
SP CD4	7·31 ± 0·34	12·48 ± 0·94	23·72 ± 0·54	5·72 ± 0·74	25·06 ± 2·84	54·16 ± 1·78	22·03 ± 4·58	38·1 ± 2·56	61·49 ± 2·73	31·68 ± 0·41
SP CD8	2·73 ± 0·64	4·44 ± 0·80	11·87 ± 3·13	5·29 ± 0·83	9·17 ± 1·16	24·58 ± 6·17	19·05 ± 2·96	11·66 ± 4·20	22·22 ± 5·85	21·27 ± 0·44

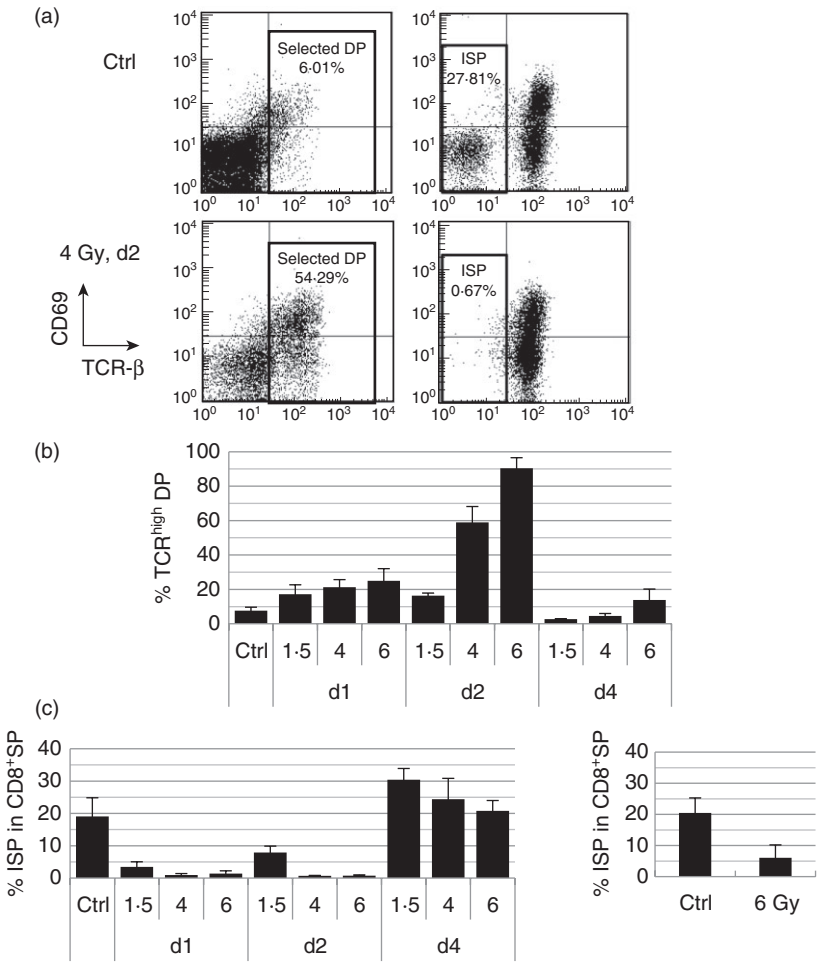

Fig. 2. Immature single-positive (ISP) and selected double-positive (DP) populations in irradiated mice. (a) Gating used to identify ISP (right) and selected DP (left) thymocyte populations from gated DP and SPCD8 + thymocytes (regions R3 and R2, respectively, in Fig. 1) in a control (top) and an irradiated (4 Gy, day 2, bottom) animal following four-colour CD4/CD8/CD69/T cell receptor (TCR)-β labelling. (b) Graph bar showing the average (± standard error) frequency of selected DP thymocytes in mice shown in Fig. 1. (c) Graph bar showing the average (± standard error) frequency of ISP in mice shown in Fig. 1 (left) and in C57BL/6 mice unirradiated and 3 h after exposure to 6 Gy (right). For the right panel, $n = 4$ for control mice, and $n = 5$ for irradiated mice.

The DP population is not homogeneous. The levels of expression of TCR and CD69 allows discrimination of $TCR^{low}CD69^{-}$ 'immature' DP from selected $TCR^{high}CD69^{+}$ and $TCR^{high}CD69^{-}$ DP, the immediate precursors of SP thymocytes (Fig. 2a). As shown in Fig. 2b, we observed a relative increase (2·2–3·2-fold over control) in the proportion of selected DP thymocytes at day 1 for all doses. This proportion is maintained at day 2 for 1·5 Gy-irradiated mice, but is increased dramatically in mice exposed to 4 Gy (7·7-fold) and 6 Gy (11·8-fold), where selected DP represent 60 and 90% of the DP population, respectively. Four days after 1·5 Gy and 4 Gy, the frequency of selected DP drops to values lower than those of control mice, whereas it is still somewhat elevated (1·8-fold) after exposure to 6 Gy. The relative increase in selected DP at days 1 and 2 shows clearly that these cells are more radio-resistant than their TCR^{low}DP precursors. The diminution of their frequency between days 2 and 4 suggests either that they take longer to die, or that they resume differentiation and progress to the SP compartment.

ISP thymocytes are very radio-sensitive

The observation that it takes 2 days for the DP population to reach its minimum following radiation exposure (Fig. 1) suggests that, in addition to DP cell death and eventual progression of selected DP to the SP compartment, the influx of ISP cells may be stopped. Indeed, we observed a very strong reduction of the frequency of $TCR^{-}CD8^{+}$ ISP cells in irradiated mice at day 1 for all doses (Fig. 2c). Whereas ISP frequency already increases at day 2 in mice exposed to 1·5 Gy, it remains at the same low level in mice exposed to higher doses. We then observed a very strong rebound in ISP frequency at day 4 for all doses to levels higher than in control animals, indicating a strong compensatory homeostatic mechanism. The frequency of ISP thymocytes was found to be reduced already by 75% 3 h after a 6 Gy irradiation (Fig. 2c), when no change in the frequency of the DN, DP and SP populations is detectable (data not shown). Thus, ISP thymocytes are more radio-sensitive than TCR^{low} DP, die earlier, but are also regenerated faster.

p53-dependency of DN2, ISP and selected DP radiation-induced apoptosis

As it is well established that DP thymocyte apoptosis requires the activity of the p53 tumour suppressor [23–25], we compared the fate of ISP and TCR^{high} DP in WT and

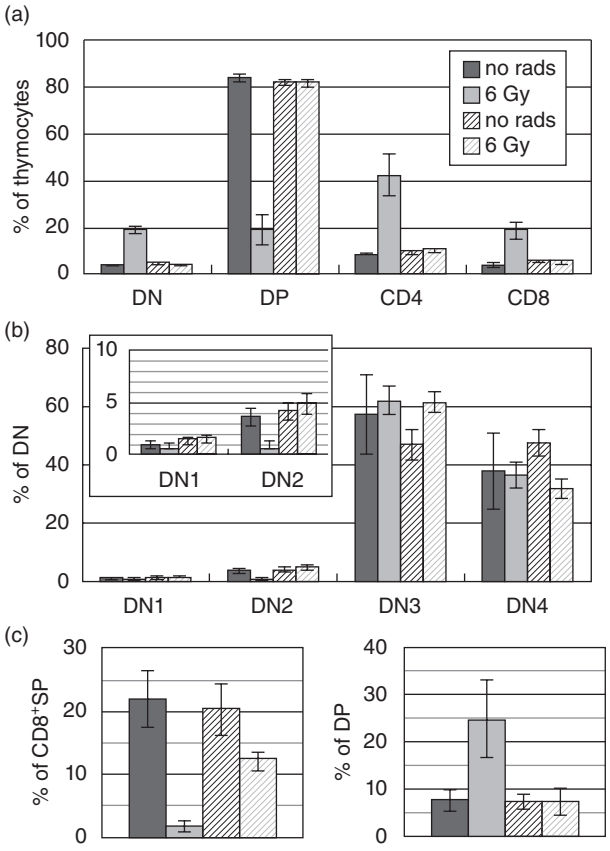

Fig. 3. Fluorescence activated cell sorter (FACS) analysis of unirradiated and irradiated (6 Gy, day 1) wild-type and p53 thymocytes. (a) Bar graph showing the average frequency (± standard error) of double-negative (DN), double-positive (DP) and single-positive (SP) populations in the indicated mice. (b) Bar graph showing the average frequency (± standard error) of DN1, DN2, DN3 and DN4 populations in the DN thymocytes of mice shown in (a). (c) Bar graph showing the average frequency (± standard error) of immature single-positive (ISP) (left) and selected DP (right) thymocytes in the SPCD8 and DP populations (regions R2 and R3, respectively, in Fig. 1) of mice shown in (a). Solid dark grey bars: unirradiated p53 wild-type, solid light grey bars: irradiated p53 wild-type, hatched dark grey bars; unirradiated $p53^{-/-}$, hatched light grey bars: irradiated $p53^{-/-}$. The number of mice in each group is as follows: $n = 6$ for unirradiated wild-type, $n = 9$ for irradiated wild-type, $n = 4$ for unirradiated $p53^{-/-}$ and $n = 6$ for irradiated $p53^{-/-}$.

p53-deficient mice 1 day after exposure to 6 Gy to determine if they share the same p53 dependency. We also included earlier immature DN thymocyte populations in our analysis.

As expected, the important diminution of DP frequency and the corresponding compensatory increase in the frequencies of DN and SP populations observed in irradiated WT mice do not take place in irradiated p53-deficient animals (Fig. 3a). However, even if the DN population, as a whole, is relatively less radio-sensitive than the DP, the DN subpopulations are differentially affected. Radiation exposure induces an 85% reduction of the frequency of $CD44^+cKit^+CD25^+$ DN2 thymocytes in WT mice (Fig. 3b, inset), indicating that they are more radio-sensitive than their DN1 precursors or their DN3/DN4 descendants. This preferential death is not observed in p53-deficient mice. As previously, we found a strong reduction of the ISP frequency in irradiated WT mice but, in contrast to DN2 thymocytes, radiation-induced ISP cell death is only partially p53-dependent, as the frequency of the ISP population in irradiated $p53^{-/-}$ mice is reduced to 60% of that found in control mice (Fig. 3c). Finally, the proportion of selected DP in the thymus of $p53^{-/-}$ mice does not change following irradiation, indicating that the higher proportion observed in irradiated WT mice results from TCR^{low} DP radio-sensitivity (Fig. 3c).

p53 is differentially expressed and up-regulated in radio-sensitive and radio-resistant thymocytes

Radiation-induced p53 activation results from phosphorylation and stabilization after dissociation from the complexes it forms with MDM2 in steady state conditions [32–34]. Hence, increased levels of phosphorylated, activated p53 protein are found in irradiated thymocytes (Fig. 4a). As DN, DP and SP thymocytes exhibit a clear difference in radio-sensitivity, we wanted to determine whether these differences would be correlated with the level of p53 up-regulation/accumulation in these cells following exposure. We analysed p53 expression by confocal microscopy in thymocytes prepared from mice 3 h after a 6 Gy irradiation (Fig. 4b). Interestingly, in thymocytes from unirradiated mice, p53 seems to be less expressed in DP than in the other populations. In thymocytes from irradiated animals we clearly observed an increase of the level of p53 expression in all populations, but it stays higher in DN and SP cells. Some apoptotic cells are also already visible, but they do not express high levels of p53.

Discussion

Altogether, our results show that high radiation doses induce a more severe thymic involution and a longer block of differentiation than an intermediate dose (1·5 Gy), which only transiently affects thymocyte development. This different behaviour most probably reflects different p53 radiation-induced levels/activation states, which result in radiation-dose-dependent transcriptional activation of pro-apoptotic p53 target genes [35]. A clearer sign of this involution is the disappearance of the prominent DP population. However, rapidly cycling ISP thymocytes, which constitute a much smaller population (approximately 20% of SPCD8 thymocytes, i.e. >1% of total thymocytes), are more radio-sensitive: their death is evident as early as 3 h post-irradiation, a time at which the DP population is not yet affected. The influx of ISP into the DP compartment is

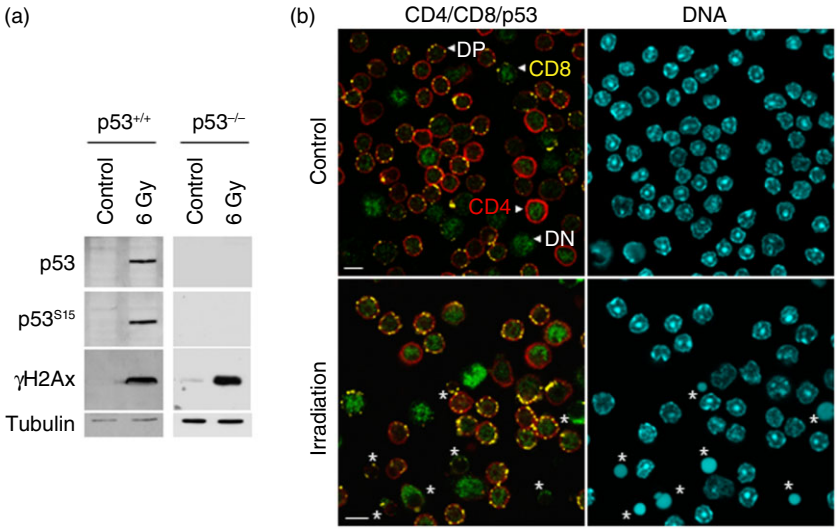

Fig. 4. Up-regulation of p53 expression in irradiated thymocytes. (a) Western blot analysis of p53 expression, p53 phosphorylation and γH2AX in thymocytes following radiation exposure of wild-type and p53-deficient thymocytes. (b) Immunofluorescent labelling and confocal microscopy of p53 expression (green) in CD4 (red) and CD8 (yellow) labelled thymocytes prepared from control (top) or 6 Gy irradiated (bottom) mice (left panels). DNA staining by 4′,6-diamidino-2-phenylindole (DAPI) (blue) is shown in the right panels. Single optical sections are shown with individual or superposed staining. The CD8 labelling is punctuated, because streptavidin-coupled Alexa 546 was used to reveal the anti-CD8 antibody binding. *Apoptotic cells; scale bar = 10 μm.

therefore arrested even before the DP thymocytes begin to die. ISP thymocytes are generated after developing thymocytes traverse the pre-TCR checkpoint. The proliferation signals received ensure a large expansion of the pool of thymocytes that succeed in rearranging and expressing a TCR-β chain and, consequently, will be available to rearrange a TCR-α chain and express a TCR-αβ in the DP compartment. The disruption of this developmental pathway by the early death of ISP therefore participates in the reduction of the number of thymocytes trying to pass positive and negative selection in a highly disturbed environment, where these selection processes may be affected by the presence of cellular debris and/or byproducts, including damage-associated molecular pattern (DAMPs) molecules, resulting from radiation-induced cell death. Interestingly, 1·5 Gy induces only a mild and very transitory thymic involution. In mice exposed to low-dose radiation, a significant fraction of DP thymocytes will therefore survive and develop in a perturbed environment, rich in DAMPs. It will be of interest to determine if the presence of these usually intracellular molecules affects DP selection and modifies the expressed TCR repertoire.

The dynamics of ISP and DP populations are not synchronous. Regeneration of the ISP compartment is already in progress at day 2 after exposure to 1·5 Gy and is massive at day 4, irrespective of the dose and the resulting level of DP involution at day 2. This kinetics probably denotes an attempt to compensate for radiation-induced DP death and replenish the DP compartment as quickly as possible, once most of the radiation effects have been eliminated. Importantly, in mice exposed to 4 and 6 Gy, this regeneration takes place in the absence of an increase in the total thymocyte number. This dichotomy suggests that there is no co-ordination of proliferation and differentiation of the different thymocyte populations (at least ISP and DP) following radiation exposure, and that the different steps of the thymocyte differentiation programme are uncoupled. In addition, the rapid restoration of the proliferation/differentiation of ISP thymocytes suggests that they are generated from intrathymic radio-resistant precursors, as described previously [36,37].

Our results also show that DP thymocytes respond differently to radiation depending on their differentiation stage. Selected TCR^{high} DP thymocytes are more radio-resistant than their unselected TCR^{low} DP precursors. The transition from TCR^{low} to TCR^{high} follows positive and negative selection of developing thymocytes. Thus, TCR signalling in DP thymocytes modulates radiation sensitivity. This may be due to epigenetic and/or transcriptional changes during differentiation to the SPCD4 or SPCD8 compartment [38]. However, DP thymocyte selection also results in down-regulation of RAG-1 and RAG-2 gene expression and the cessation of V(D)J recombination [39–41]. Hence, when their TCR specificity is fixed and they are no longer performing 'dangerous' genomic rearrangements requiring the generation of 'physiological' DSBs in a controlled setting [13,16–18], DP thymocytes become less prone to die when more DSBs are generated by radiation. One interpretation could be that rearranging TCR^{low} DP are 'sensitized' to DSBs, i.e. that their DSB checkpoints are pre-activated [20–22,42] and, therefore, they might be more susceptible to initiate apoptosis when additional radiation-induced DSBs are generated. The finding that DN2 thymocytes, the population in which TCR-β gene rearrangement is initiated [26–28], is more radio-sensitive than the other DN populations supports this hypothesis. We would therefore like to propose that the high level of radio-sensitivity observed in DN2 and TCR^{low} DP thymocytes is correlated to their V(D)J recombination activity.

Finally, it is well known that radiation-induced p53 activation and its outcomes, i.e. induction of apoptosis, cell cycle arrest or senescence, varies largely according to the tissular and/or cellular context [43,44], probably reflecting both the cell-specificity of radiation-induced p53 activation

and extracellular survival/differentiation signals received by the irradiated cells. Bax and p21 proteins were, for example, found to be induced differentially in the thymus of irradiated mice (5 Gy). Bax was more induced in the medulla, the anatomical location of mature SP thymocytes, than in the cortex, where DN and DP thymocytes reside and, even though both Bax and p21 induction were heterogeneous, the patterns of their induction were different. This work illustrates the specificity of p53 transcriptional activity in the same organ [45]. In this study, we now show that whereas DN2 and TCR^{low} DP, both actively rearranging TCR genes, undergo p53-dependent apoptosis following radiation exposure, p53-independent radiation-induced cell death occurs in ISP (Fig. 3). The high proliferation rate of ISPs cannot explain this different behaviour, as DN2 are also cycling [46,47]. This difference in p53 dependence may reflect changes in pro-apopotic/survival factors resulting from pre-TCR signalling [48–50]. p53 has been proposed to enforce thymocyte differentiation checkpoints [20,51,52], and its activity has been shown to be regulated by different pathways in developing thymocytes [50,53]. We now show that the basal level of p53 expression is regulated developmentally during thymocyte maturation in unirradiated mice. Interestingly, it is lower in DP than in the other populations. Radiation induces up-regulation of p53 expression as early as 3 h post-exposure in all the populations, but remains lower in DP thymocytes than in DN and SP cells. Thus, there is clearly no correlation between radiosensitivity and the level of p53 expression, as those cells that will preferentially survive express the highest level of p53, even if all thymocytes were exposed to the same dose of genotoxic radiation. This higher level of p53 expression was already present in the steady state. Our results showing that DP radio-sensitivity is regulated by TCR signalling underscore the crucial importance of the cellular context in the determination of cell fate following radiation exposure.

A similar phenomenon can also be observed in peripheral T cells: apoptosis induction is different in quiescent human naive, effector, central memory and effector memory CD4 and CD8 human T lymphocytes irradiated *ex vivo* [54]. It is therefore likely that, *in vivo*, these different T lymphocyte subsets will also respond differently to radiation exposure, for example during radiotherapy. In this situation, an eventual difference in radio-sensitivity between effector and regulatory T cells (T_{regs}) could result in critical changes in the tumour-specific immune response. In tumour-bearing mice, T_{regs} were found to be relatively more radio-resistant, and therefore enriched both *in situ* and in the spleen, following irradiation (10 Gy) of the tumour [55]. In apparent contradiction with these findings, preoperative radiotherapy was found to promote a local increase in proliferating $CD8^+$ T cells [56] and radiochemotherapy to lead to a higher cytotoxic/forkhead box protein 3 ($FoxP3^+$) T cell ratio due to a decrease in regulatory T cells [57] in patients with head and neck cancer. T_{regs} were also found to be the more affected intratumoral lymphocyte subset in oesophageal cancer following radiochemotherapy [58,59]. Therefore, in these studies, cumulative doses around 50 Gy, with the eventual administration of chemotherapeutic drugs, result in a relative decrease in T_{regs}. Several factors affecting one or more lymphocyte subsets including, for example, preferential apoptosis, activation/proliferation or intratumoral recruitment may contribute to this effect, and more studies will be needed to determine the role, if any, of differential radio-sensitivity on the dynamics of intratumoral lymphocytes following local irradiation.

Disclosure

None.

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